Effect of Scorpion Venom on the Ciliary Activity of Fresh Water Mussel

BY

D. CHENGAL RAJU, R. V. KRISHNAMOORTHY AND A. SUBBARAMI REDDI

Department of Zoology, Sri Venkateswara University, Tirupati, A. P., India

(2 Text figures)

ALTHOUGH NUMEROUS investigations have been conducted on the chemical make-up of the venoms of several scorpions, comparatively scanty information is available on the biochemical and physiological effects of these venoms. Preliminary studies of OOMMEN *et al.* (1964) have shown that the venom of the South Indian scorpion, *Heterometrus scaber,* inhibits strongly the activity of lactate, citrate and succinate dehydrogenases of frog muscle. Similar results were obtained on the dehydrogenases of cockroach muscle (BABU *et al.*, 1971). In the present investigation an attempt was made to study the effects of the venom of another South Indian scorpion, *Heterometrus fulvipes,* on the ciliary activity of fresh water mussel, since the ctenidia of this bivalve are known to be involved in the respiration and feeding of the animal. The venom of this species of scorpion is of special interest because of its deleterious effects on several invertebrates and vertebrates, including human beings. The choice of this material was made because of direct visual observation of the effects of the venom on the ciliary activity.

MATERIAL AND METHODS

The scorpions, *Heterometrus fulvipes,* were collected from the surrounding places of the University Campus. The venom was extracted by electrical excitation as follows: one electrode was placed in contact at the telson joint and the other at the arthrodial membrane of the chelate leg. Make and break stimuli were applied with a dry battery of 1.5 volt in circuit. Venom collected as a result of this electric excitation at the tip of the sting was removed with a pipette.

A complete ctenidium of the fresh water mussel, *Lamellidens marginalis,* was isolated and fixed on a wax-laden finger bowl. This preparation was kept in pond water. Ciliary activity was observed by the movement of a fine film strip bit placed at the edge of the gill as recommended by WELSH & SMITH (1960). The time taken for the film strip to be dragged of 1 cm of the length of the gill was noted. The reciprocal of time was taken as the measure of the ciliary rate. The experiment was repeated with the natural medium at temperatures of 35°, 30° and 25° C. Temperature was maintained by keeping the finger bowl in a water bath with the desired temperature. The same film strip was used for all the experiments. The data were subjected to statistical validation.

The medium with venom added was prepared at a concentration of 0.01 ml venom per 10 ml pond water. Per cent changes in the ciliary rate in the medium with venom and the venom-free medium were calculated over that in the normal medium, considering the latter as 100%. Logarithmic dilutions were made with pond water. For the recovery experiments, venom-free medium was prepared by replacing the pond water with venom added with natural pond water after washing the preparation thoroughly with pond water.

The magnitude of the effect of the venom at different temperatures was evaluated through Q_{10} (PROSSER & BROWN, 1961).

RESULTS AND DISCUSSION

It is clear from Table 1 that the venom of the scorpion in general inhibited the rate of ciliary activity. After the addition of the venom the activity decreased by about 21% and when all the venom was removed by replacing it with fresh pond water the restoration of activity to normalcy falls short by only 9% (Table 1). This experiment shows that evidently the venom causes reversible inhibition as long as it is present in the medium and when it is removed, the original activity is restored. Figure 1 illustrates the rate of ciliary beat effected by the dilution

Table 1

Changes in the ciliary rate of fresh water mussel in media with venom added (0.01 ml/10 ml) and in venom-free media

Environment	Number of observations	% ciliary rate after 10 min. at 30° C over normal
Normal (pond water)	12	100.00
Venom added (inhibition experiment)	12	79.00±4.48
Venom-free after thorough washing the preparation with pond water (recovery experiment)	12	91.00±2.30

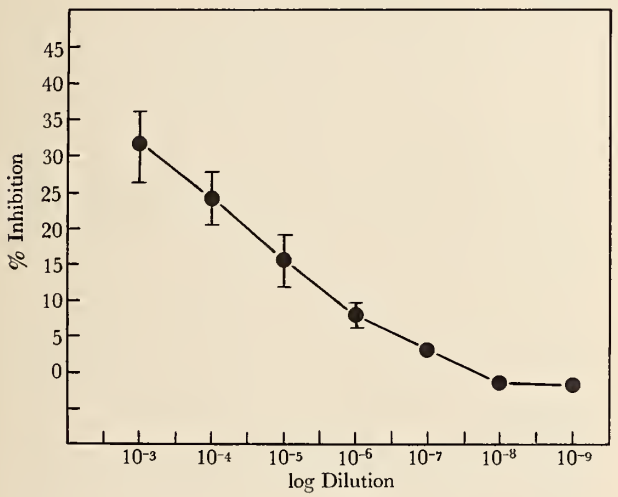

Figure 1

of the venom. As is evident from the figure the greater the dilution the less was the inhibition and finally the dilution over 10^{-8} would not inhibit the ciliary activity to any detectable degree.

The rate of movement of cilia on the addition of venom in relation to temperature is given in Figure 2. At all temperatures the general trend of the ciliary activity in the medium with venom added remained the same, but the magnitude of activity varied with the temperature. The rate of activity decreased with increasing temperature, indicating that the venom is more active at higher temperatures. It appears probable that increase of temperature facilitates the faster diffusion of the venom into the cytoplasm resulting in the decreased activity of cilia.

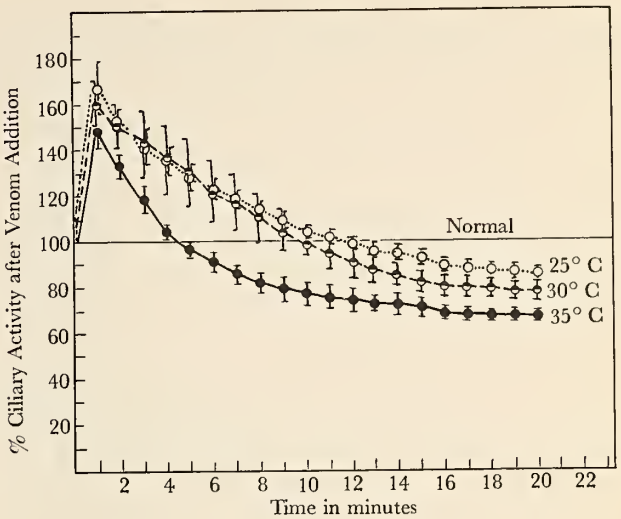

Figure 2

Hence it is suggested that the inhibition of ciliary beat by the venom is temperature dependent. In order to know the magnitude of the effect of temperature on ciliary rate in medium with venom added, Q_{10} values were calculated for the temperature ranges at which the ciliary activity was studied. It is evident from Table 2 that the Q_{10} values for both higher and lower ranges of temperature are not similar and are statistically insignificant. As Q_{10} is the measure of the rate of reaction (Prosser & Brown, 1961) and as it is the same in the higher and lower ranges of temperature studies, the rate of ciliary function changes in the same magnitude over the normal on both extremes of temperature range studied in the medium with venom added.

Table 2

Q_{10} values of the rate of ciliary activity in medium with venom added (0.01 ml/10 ml)

Temperature range 25 - 30° C	Temperature range 30 - 35° C	t test (n = 7)	Change in Q_{10} values at higher ranges of temperature
1.17±0.1307	1.29±0.118	t = 0.2894	Insignificant since P > 0.05

There was an outburst of ciliary activity immediately after the introduction of the medium with the venom added (Figure 2). This overshoot response then gradually

diminished and the real inhibition due to the presence of the venom in the environment was established at every temperature. It is clear from the diagram (Figure 2) that the outburst of activity was more at 25° C and less at 35° C. The overshoot response observed here may be compared with that described by GRAINGER (1958), according to whom this response is the result of a feed back control to the immediate change in the natural habitat with the addition of toxins. In order to resist this toxicity, the system accelerates its activity but apparently fails to maintain it due to the over-impeding forces of toxicity, which finally cause inhibition.

The inhibition of ciliary activity in the presence of venom may be explained on the permeability properties of the cell membranes also. It is known that toxins alter the permeability of the cell membranes besides other effects on physiological processes (ABBOTT & BALLANTINE, 1957; BERGMANN *et al.*, 1963; PARNAS, 1963). Hence it is suggested that the scorpion venom may contain several toxic substances which affect the permeability of the ciliary membranes, resulting in the decreased activity of the cilia. Since the main function of ctenidia in the fresh water mussel is respiration, it appears quite likely that the scorpion venom inhibits the respiratory metabolism of the animal also. Further studies along these lines are worth attempting.

Another interesting feature observed during this investigation was the excessive secretion of mucus over the gill fragments when the venomous medium was added. But the cause for this is unknown.

SUMMARY

1. The rate of ciliary activity of fresh water mussel was studied at different temperatures in the environment of scorpion venom. The activity was found to be inhibited by the venom; the degree of inhibition was found to be dependent on the dilution factor.
2. The onset of inhibition was dependent on time and temperature.
3. An overshoot response was observed immediately after exposing the cilia to the environment with venom added. The possibility of scorpion venom altering permeability of the ciliary membranes was discussed.

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